Effect of respiratory syncytial virus on subsequent allergic sensitization to ovalbumin in guinea-pigs

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ABSTRACT: Children with acute respiratory syncytial virus (RSV) bronchiolitis often develop recurrent wheezing, asthma and allergic sensitization, but the role of RSV in the pathogenesis of these sequelae is unclear. This study examined whether RSV infection potentiates subsequent allergic sensitization, airway hyperresponsiveness (AHR) and airway inflammation induced by repeated exposures to aerosolized ovalbumin (OA) in guinea-pigs.

Guinea-pigs received either RSV or sham inoculum, followed by exposures to OA- or saline-containing aerosols to form the following groups: 1) noninfected, nonsensitized controls (sham/saline group); 2) RSV-infected, nonsensitized animals (RSV/saline group); 3) noninfected, OA-sensitized animals (sham/OA group); 4) RSV infection and first OA exposure on the same day (RSV/OA group), and 5) RSV infection six days prior to first OA exposure (RSV6/OA group). Three days after the final aerosol exposure, circulating OA-specific immunoglobulin (Ig)G_1 antibody titres and AHR to inhalation acetylcholine challenge were measured and morphometry performed to evaluate allergic inflammation of the airways.

OA-exposed animals developed OA-specific IgG$_1$ antibodies, AHR and airway eosinophilia (sham/OA, RSV/OA and RSV6/OA groups. RSV infection alone induced significant AHR and airway eosinophilia (RSV/saline group). RSV infection, and concomitant exposure to OA (RSV/OA group) enhanced OA-specific IgG$_1$ antibodies, but not airway eosinophilia or AHR. Such increases were not observed in the RSV6/OA group.

In conclusion, respiratory syncytial virus potentiates the production of ovalbumin-specific immunoglobulin G$_1$ antibodies in guinea-pigs, but circulating titres of these antibodies do not reflect the extent of airway hyperresponsiveness or airway inflammation. In addition, respiratory syncytial virus infection alone can produce slight increases in airway hyperresponsiveness that are associated with increased numbers of eosinophils in the airways.
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Respiratory syncytial virus (RSV) infection is the most common cause of acute bronchiolitis affecting children <2 yrs of age [1]. Children hospitalized for acute RSV bronchiolitis frequently develop sequelae of recurrent wheezing and asthma [2] that may relate to a genetic predisposition [3]. Viral respiratory tract infections involving RSV, parainfluenza virus (PIV) and cytomegalovirus have also been implicated in the onset of allergic sensitization in children born into families with a history of allergy [4]. By contrast, other investigators have postulated that viral respiratory tract infections during early postnatal life may be protective against the development of asthma or allergy [5, 6]. Overall, there is considerable controversy with regard to the potential role of viral respiratory tract infections, including acute RSV bronchiolitis, in triggering allergic sensitization, or potentiating airway hyperresponsiveness (AHR) and/or airway inflammation that are characteristic of asthma [7].

The interaction between viral respiratory tract infection and allergic sensitization has been studied in a number of animal models. Early studies in mice [8, 9] examined the effects of influenza virus infections on the production of ovalbumin (OA)-specific immunoglobulin (Ig)E antibodies in animals that were subsequently sensitized to OA. The results of these experiments suggested that the inflammatory response developing during an acute, lytic viral infection could be necessary for the production of OA-specific IgE. Similar studies have reported that acute RSV infection of mice potentiated the production of allergen-specific IgE [10, 11]. Recent studies of PIV-infected guinea-pigs [12] and RSV-infected mice [13] attempted to expand upon these findings by including measurements of AHR and lung inflammation, in addition to viral effects on production of OA-specific IgE and/or IgG$_1$ (the major subclass of antibody involved in the allergic responses of guinea-pigs [14]). However, the results of these studies showed apparent discrepancies in the effects of antecedent viral infection on antibody production, AHR and lung inflammation in animals subsequently sensitized to OA. Specifically, acute PIV-3

infection of guinea-pigs before OA sensitization resulted in increased production of OA-specific IgG_1, specific AHR to OA challenge, and increased numbers of eosinophils recovered by bronchoalveolar lavage (BAL) [12], while RSV-infected mice showed increases in AHR and inflammation, without concomitant increases in OA-specific IgE titres [13]. The relationship between circulating titres of OA-specific IgE/IgG_1, AHR and lung inflammation remains unclear.

In this laboratory, guinea-pigs have been used as an animal model of allergic sensitization to OA [15] and as a model of acute RSV bronchiolitis [16, 17]. In this animal model, repeated exposures to aerosolized OA results in the production of OA-specific IgG_1 antibodies, nonspecific AHR to inhaled acetylcholine (ACh), and increased numbers of eosinophils within the airways [15]. Experimental inoculation of human RSV into juvenile guinea-pigs produces histological features of human acute bronchiolitis, in which bronchiolar inflammation and AHR are maximal at 6–7 days after RSV inoculation [16, 17]. It has recently been reported that acute RSV infection increases AHR in guinea-pigs that were presensitized to OA [18], but the effect of acute RSV infection prior to OA exposure on OA-specific IgG_1 production, AHR or airway inflammation is not known.

The purpose of this study was to investigate the effects of acute RSV infection on the subsequent allergic sensitization to aerosolized OA in juvenile guinea-pigs. The experimental approach consisted of measuring circulating titres of OA-specific IgG_1 antibodies and AHR, and performing airway morphometry to determine the numbers of infiltrating eosinophils and metachromatic cells (mast cells/basophils) in multiple airway compartments.

Methods

Study design

The experimental design is illustrated in figure 1. Forty guinea-pigs were randomly assigned into five groups (n=8 for each group). Three groups of guinea-pigs were inoculated with RSV and either exposed immediately to aerosols containing saline (RSV/saline group) or 1% OA in saline (RSV/OA group), or exposed six days after the infection to aerosols of 1% OA (RSV6/OA group). The two intervals between RSV infection and first exposure to OA were chosen to correspond to times preceding or concomitant with virus-induced acute bronchiolar inflammation [16]. Uninfected guinea-pigs were similarly inoculated with uninfected (sham) cell culture medium and subsequently exposed to ovalbumin (sham/OA group) or saline (sham/saline group) (fig. 1). Following a sensitization period of 7 days, the animals were challenged three times per week for two consecutive weeks with aerosols containing 0.5% OA or saline, depending on the group. Three days after the last aerosol challenge, the animals were injected with pentobarbitone (*i.p.*) and exsanguinated by cardiac puncture. Specimens of serum were prepared from whole blood for titration of OA-specific IgG_1 antibody, and the lungs were processed for viral culture and for histological analyses of the allergic inflammatory cell response. For lung mechanics measurements, another set of 40 guinea-pigs were studied, in which ACh chal-
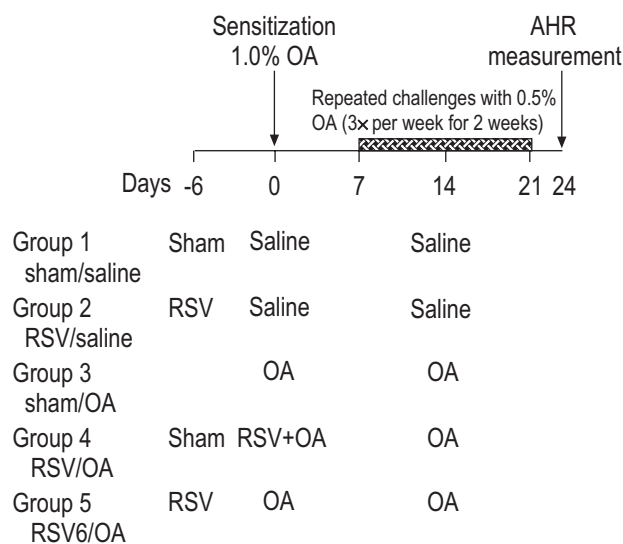

Fig. 1. – Schematic representation of experimental design. Airway hyperresponsiveness (AHR) measurement and the collection of blood and lung tissue specimens occurred 3 days after the last aerosol exposure. OA: ovalbumin; RSV: respiratory syncytial virus; RSV6: RSV 6 days prior to OA. AHR was to acetylcholine challenge.

lenge was performed 3 days after the last aerosol exposure. A separate set of animals were used because it has been shown that the ACh challenge protocol can affect airway morphometry in guinea-pigs by producing artefact, thereby yielding potentially misleading results [19]. All experiments were performed with the approval of the Animal Care Committee at the University of British Columbia.

Virus preparation

Human Long strain, type A RSV (American Type Culture Collection (ATCC), Rockville, MD, USA) was propagated on HEp-2 (ATCC) cell monolayers as previously described [16]. The culture medium consisted of minimal essential medium (MEM); Gibco, Grand Island, NY, USA) supplemented with 2% heat-inactivated foetal bovine serum (FBS) (Hyclone Laboratories Inc., Logan, UT, USA), 0.292 $mg \cdot mL^{-1}$ L-glutamine (Gibco) and 50 $\mu g \cdot mL^{-1}$ of gentamicin (Gibco). After five days of culture, the culture fluids containing free virus were harvested and centrifuged at $10,000 \times g$ for 10 min at $4^{\circ}C$ to remove cell debris. The clear supernatants were then pooled and concentrated by ultrafiltration through a 100 kDa cut-off membrane (Centriprep-100; Amicon Corp., Toronto, ON, Canada) according to the manufacturer's instructions. Viral stocks were kept at $-70^{\circ}C$ and used for inoculation of guinea-pigs within one week of storage. Supernatants of uninfected HEp-2 cell cultures were similarly prepared for sham inoculation of control animals.

Inoculation protocol

Female Cam-Hartley guinea-pigs (1 month-old, body weight: 250–300 g) were purchased from Charles River Laboratories (Montreal, QC, Canada) and housed in filter-isolated large polycarbonate cages and acclimatized for

one week before experimentation. Animals were lightly anaesthetized by inhalation of 5% halothane and inoculated intranasally with 200 µL of diluted RSV suspension (4.5×10^{-4} plaque forming units (pfu)·animal^{-1}), pre-warmed at 37°C, as previously described [16]. Uninfected, control guinea-pigs were similarly anaesthetized and given the same volume of sham inoculum. The infected animals were housed in a different room from the uninfected animals, but were otherwise maintained under identical conditions of alternating 12 h light-dark cycles, and free access to guinea-pig chow (Ralston Purina Corp., St. Louis, MO, USA) and water.

OA sensitization and challenge

Guinea-pigs were placed in polycarbonate chambers and sensitized for 10 min by inhalation exposure to an aerosol of 1% OA solution (grade III, Sigma Chemicals, St. Louis, MO, USA) in sterile 0.9% saline, delivered by an ultrasonic nebulizer (DeVilbiss, Sunrise Medical, Somerset, PA, USA) as previously described [15]. Adjuvant was not used because in preliminary experiments (not shown), the findings of SUGIYAMA et al. [20], who documented that successful sensitization to aerosolized OA could be achieved in guinea-pigs without the requirement for an adjuvant, were confirmed. Aerosol challenges commenced 7 days after the initial sensitization procedure and consisted of 10 min exposures to 0.5% aerosols of OA in 0.9% saline, repeated three times per week for two consecutive weeks. All animals were pretreated with diphenhydramine (40 mg·kg of body weight^{-1} i.p.), 20 min prior to OA challenge, to prevent anaphylactic death.

OA-specific immunoglobulin G_1 titres

Circulating titres of anti-OA IgG_1 antibodies were determined in each guinea-pig's serum by using an enzyme-linked immunosorbent assay (ELISA), derived from a previous method [21]. Briefly, polypropylene 96-well microtitre plates (Falcon, Mississauga, ON, Canada) were precoated overnight at 4°C with 5 µg OA·well^{-1} in carbonate/bicarbonate coating buffer (Sigma) and washed four times with Tween-containing, phosphate-buffered saline (PBS-T; 10 mM PBS, pH 7.4 with 0.05% Tween-20). Guinea-pig sera were serially two-fold diluted (1:50–1:1,600 in PBS-T with 1% bovine serum albumin (BSA; Sigma)) and 100 µL were added to each well for 2 h of incubation at room temperature. Samples were washed four times with PBS-T before addition of 200 µL of optimally diluted (1:2,000 in PBS-T +1% BSA) peroxidase-conjugated goat anti-guinea-pig IgG_1 (Nordic Immunological Laboratories, Capistrano Beach, CA, USA). This antibody recognizes the Fc portion of the IgG_1 molecule and does not cross-react with other components of the immunoglobulin system or with other proteins present in the guinea-pig's serum. After 2 h of incubation, the wells were washed four times and incubated with 200 µL of o-phenylenediamine dihydrochloride (OPD) peroxidase substrate (Sigma) for 30 min. This enzymatic reaction was terminated by the addition of 50 µL 3M H_2SO_4 and the optical density (OD) was determined at a wavelength of 492 nm in an ELISA microtitre plate reader (Titertek Multiskan Plus MK2; Titertek, Huntsville, AL, USA). Titres were determined as the greatest dilution of serum to produce an OD of at least 0.1 U above control values. Controls consisted of sera obtained from unexposed, naive animals. This endpoint of 0.1 OD units above control values was predetermined from point titration of the least amount of guinea-pig IgG that can be detected by the method using the same optimal dilution of anti-guinea-pig IgG_1. At this cut-off level, the lower limit of detection was 1.5–2 times higher than background values.

Measurement of lung mechanics

The protocol for measurement of lung mechanics by challenge with inhaled ACh was based on previous work in OA-sensitized and RSV-infected guinea-pigs [15, 17, 18]. Three days after completion of the last aerosol challenge, each guinea-pig was anaesthetized by i.p. injection of ketamine (50 mg·mL^{-1}) and xylazine (10 mg·mL^{-1}). A catheter was inserted through the mouth into the distal oesophagus of the guinea-pig which was cannulated via the trachea and then placed into a whole-body plethysmograph. All guinea-pigs were paralysed with the neuromuscular blocking drug, succinylcholine, and ventilated with a small animal ventilator (Harvard Instruments Corporation, South Natick, MA, USA) at a rate of 60 breaths·min^{-1} and a tidal volume of 10 mL·kg of body weight^{-1}. Pulmonary resistance (RL) was measured at baseline, following nebulized saline, and after each dose of ACh (0.5, 1.5, 5, 15 and 50 mg·mL^{-1}). Acetylcholine was administered as six tidal breaths with a flow rate of 8 L·min^{-1}. Following each dose of ACh, it was ensured that the transpulmonary pressure had returned to baseline prior to the next dose. RL was calculated for the baseline, 15 and 50 mg·mL^{-1} doses of ACh, since in previous studies it has been shown that there were no significant differences between groups of OA-sensitized, RSV-infected or control guinea-pigs at doses of ACh <15 mg·mL^{-1} [15, 17, 18]. RL was calculated from the electrically differentiated volume signal and the transpulmonary pressure according to the method of NEERGAARD and WIRZ [22], by using a DIREC physiological recording system incorporating ANADAT (Raytech Instruments, Vancouver, BC, Canada) and expressed as the absolute change in pulmonary resistance (cmH$_2$O·mL^{-1}·s).

Airway morphometry

Airway inflammation was evaluated by histological examination of paraffin-embedded lung tissue sections, by counting the numbers of eosinophils and metachromatic cells in three airway compartments (mucosa, submucosa and adventitia), and normalized to the airway size, as outlined by BAI et al. [23]. The left lung was inflated with a 1:1 (v/v) mixture of optimal cutting temperature compound (Miles Scientific, Elkhart, IN, USA) and 4% paraformaldehyde in PBS and longitudinal slices were cut with a scalpel blade to allow for cross-sectioning of a maximum of airways. These slices were then placed in tissue cassettes and fixed for an additional 24 h in 4% paraformaldehyde in PBS, before processing into

paraffin. Serial, 5-μm sections were cut from each paraffin block and stained with: haematoxylin and eosin for routine histological examination; Masson′s trichrome stain for morphometric measurement of the perimeter of the epithelial basement membrane of the airway (Pbm); Hansel′s stain to identify eosinophils; and toluidine blue stain to identify metachromatic cells (mast cells/basophils) [24]. Slides were coded such that the observers were not aware from which group of guinea-pigs a given slide originated.

The Bioquant IV software image analysis system (R & M Biometrics, Nashville, TN, USA) was used for airway morphometry. Up to 10 small-to-midsized, noncartilaginous airways (Pbm range: 0.25–1.25 mm) were studied per lung section. Eosinophils and metachromatic cells infiltrating the same airway were counted on consecutive sections, and their numbers were expressed per mm of Pbm. These numbers were then averaged for each animal and the mean values for each group underwent a square root transformation for statistical comparisons.

Viral plaque assay

In order to assess successful lung infection by RSV and to determine the amounts of intrapulmonary, replicating virus for each infected animal, the right lung was weighed and homogenized in 3 mL of MEM. The homogenate was further sonicated on ice for 1 min at the maximum output from a Vibra cell sonicator (Sonics & Materials Inc., Danbury, CT, USA). The resulting lysate underwent centrifugation for 15 min at $1,500 \times g$ to remove cell debris, and the clear supernatant was used to determine the amounts of replicating virus by plaque assay on HEp-2 cells, as previously described [16]. The results were expressed as numbers of viral $pfu \cdot g$ of lung tissue^{-1}.

Statistical analyses

The data are presented as mean±SEM. Data for the OA-Specific IgG$_1$ antibody titres were normalized by geometric transformations (Log$_2$). Mean R_L values between the five groups of guinea-pigs were compared by using multivariate, repeated measures analysis of variance (ANOVA), and a sequential rejective Bonferroni procedure was used to correct for multiple comparisons [25]. All other measurements underwent square root transformation and were analysed by using a one-way ANOVA with nesting. A p-value <0.05 was considered to be statistically significant.

Results

No differences for either initial or final body weights were present between the five groups of guinea-pigs. Infectious RSV was recovered from the lungs of all of the infected animals, and the mean numbers of intrapulmonary, replicating virus were similar between the RSV/saline, RSV/OA and RSV/OA groups (table 1).

OA-specific antibody titres

OA-specific IgG$_1$ antibodies were detected in the sera of all guinea-pigs that underwent repeated exposures to aerosolized OA. The titres of OA-specific IgG$_1$ were significantly higher in the RSV/OA group than in the sham/OA group (p<0.05). The RSV6/OA group showed intermediate values. No OA-specific IgG$_1$ antibodies were detected in sera obtained from the RSV/saline or sham/saline groups (table 1).

Lung mechanics

Table 2 shows that at a dose of ACh of 50 mg mL^{-1}, R_L was significantly increased in all treatment groups in comparison to the sham/saline controls. The greatest increases in R_L were observed in the three groups of animals that were exposed to aerosolized OA, irrespective of whether animals were infected with RSV. Interestingly, at the ACh dose of 50 mg mL^{-1}, the RSV/saline group had significantly higher R_L than the sham/saline control group, indicative of an increase in AHR from RSV infection alone.

Airway morphometry

Figure 2 shows representative photomicrographs of Hansel- and toluidine blue-stained sections of guinea-pig airways. Table 3 shows the results of eosinophil and metachromatic cell counts in the airways of the five groups of animals studied. There were significant increases in the numbers of eosinophils in all three airway compartments and increases in the numbers of adventitial metachromatic cells in the sham/OA, RSV/OA and RSV6/OA groups, in comparison to the RSV/saline and sham/saline groups. In addition, the RSV/saline group had significantly more eosinophils in the adventitial and submucosal compartments than the sham/saline controls group. Statistical analysis of the numbers of metachromatic cells in the mucosal or submucosal compartments of the airways was precluded owing to very low numbers of these cells (<0.05 cells·mm Pbm^{-1}) observed in any of the five groups of guinea-pigs studied.

Table 1. – Viral plaque assays and circulating titres of ovalbumin (OA)-specific immunoglobulin (Ig)G$_1$

	Sham/ saline	RSV/ saline	Sham/ OA	RSV/ OA	RSV6/ OA
Lung viral titres pfu·g lung tissue^{-1}	ND	155±19	ND	143±15	133±17
Circulating titres of OA-specific IgG$_1$	ND	ND	525±84	950±73*	680±114

Data for plaque assays are presented as mean±SEM. Data for circulating OA-specific IgG$_1$ are presented as mean reciprocal titre±SEM. ND: not detected; RSV6: respiratory syncytial virus 6 days prior to OA. *: p<0.05, significantly different for RSV/OA *versus* sham/OA.

Table 2. – Measurements of lung mechanics

Treatment group	R_L responses to ACh cmH$_2$O·mL^{-1}		
	Baseline	15 mg·mL^{-1}	50 mg·mL^{-1}
Sham/saline	0.16±0.01	0.89±0.15	1.40±0.13
RSV/saline	0.15±0.01	1.16±0.16	2.20±0.20*
Sham/OA	0.18±0.04	1.71±0.63	3.04±0.70*
RSV/OA	0.16±0.03	1.10±0.39	2.78±0.44*
RSV6/OA	0.16±0.01	1.28±0.29	3.30±0.70*,+

Data are presented as mean±SEM. R_L: pulmonary resistance; ACh: acetylcholine; RSV: respiratory syncytial virus; OA: ovalbumin; RSV6: RSV 6 days prior to OA. *: $p < 0.05$, significantly different from sham/saline group; +: $p < 0.05$, significantly different from RSV/saline group.

Discussion

Allergic airway inflammation and AHR are important characteristics of asthma [7]. Clinical and experimental observations have implicated respiratory viral infections in early postnatal life in the development of airway sensitization to aeroallergens and AHR in young children [2–4, 8, 9, 12, 13]. The present study investigated the effect of RSV infection on subsequent sensitization to inhaled OA and AHR in a guinea-pig model of experimental RSV bronchiolitis and allergic sensitization to aeroallergen.

The results showed that repeated inhalation exposures of naive guinea-pigs to aerosolized OA resulted in the development of OA-specific IgG$_1$ antibody (the functional equivalent to mouse IgE [14]), airway eosinophilia and AHR. The OA-specific antibody response was further increased in guinea-pigs infected with RSV and concomintly exposed to aerosolized OA. However, no such enhanced OA-specific antibody response was observed when sensitization was performed 6 days post-RSV infection, when maximal virus-induced lung inflammation is usually observed [16].

The findings of this study are in agreement with results of previous studies of mice that were sensitized to OA after influenza A virus infection. In one of these studies, titres of OA-specific IgE progressively decreased when antigen exposure occurred at 6–8 days postinfection [8], while another study showed no significant viral-enhancement of OA-specific IgE when first OA exposure occurred at 7 days postinfection [9]. Possible explanations to the apparently critical timing of antigen exposure relative to viral infection may include temporal differences in the amount of antigen deposited in the airway mucosa, factors related to the uptake and processing of antigen, and/or the local immune environment. In RSV-infected guinea-pigs, airway inflammation is maximal at 6–7 days postinfection and is characterized by a prominent airway infiltration by phagocytes such as monocytes and granulocytes [16, 21]. These phagocytes may be involved in the clearance of foreign antigens deposited on the surface or within the airway mucosa, thereby limiting the amount of antigen available for airway sensitization. Further studies are required to determine the mechanisms by which the timing of viral infection and antigen exposure could influence airway sensitization.
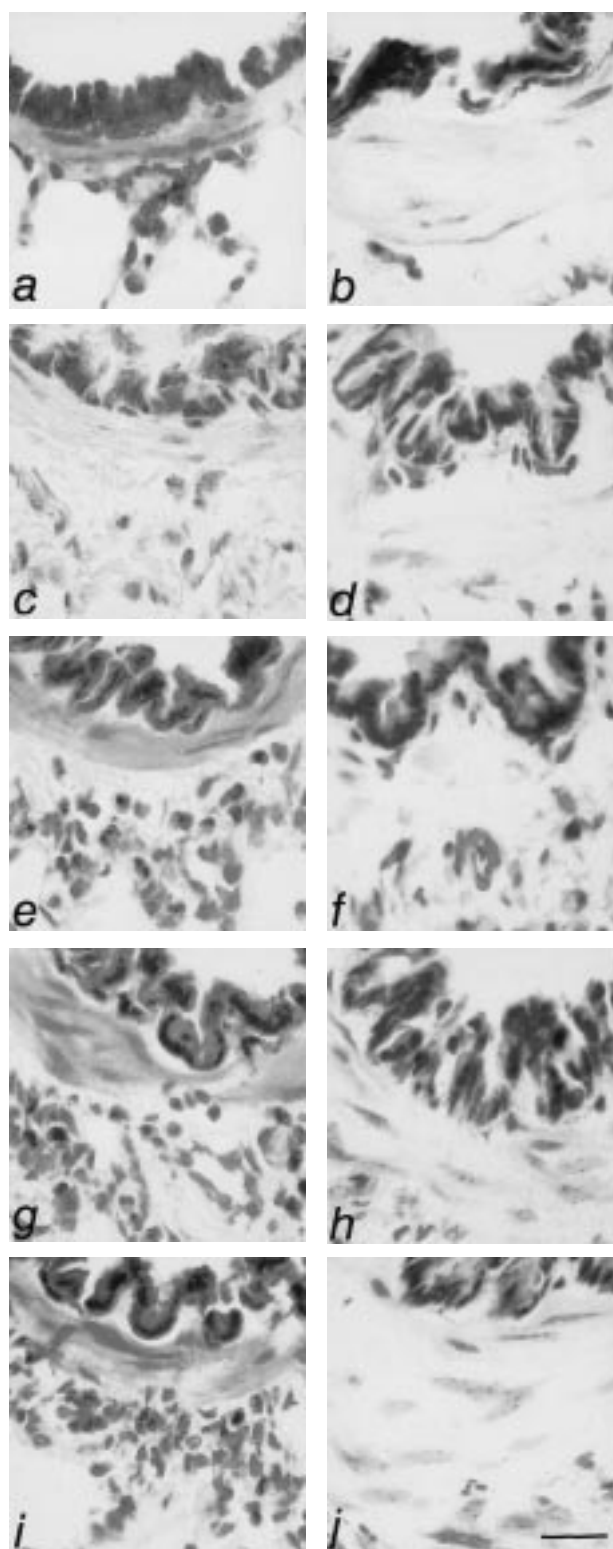

Fig. 2. – Photomicrographs showing Hansel-stained (a, c, e, g, i) and toluidine blue-stained (b, d, f, h, j) sections of guinea-pig airways. Note the prominent eosinophil infiltrates (red-staining cells) within the airways of the sham/ovalbumin (OA) (e, f), respiratory syncytial virus (RSV)/OA (g, h) and RSV6/OA (i, j) groups; the airway from a RSV/saline (c, d) animal has eosinophil infiltrates intermediate between those observed in the sham/saline (a, b) control group and in the three groups of OA-sensitized animals. In all groups of animals, there are rare metachromatic (magenta-staining) cells within the adventitia. (Internal scale bar = 25 μm.)

Table 3. – Airway inflammatory cell response

	Sham/saline	RSV/saline	Sham/OA	RSV/OA	RSV6/OA
Eosinophils					
Adventitia	10.3±1.8	14.2±1.6*	62.8±6.1[+]	66.5±5.4[+]	51.0±4.4[+]
Submucosa	1.1±0.5	2.6±0.8*	11.5±1.9[+]	11.5±1.9[+]	7.7±1.6[+]
Mucosa	0.0	0.1±0.1	1.4±0.3[+]	1.5±0.3[+]	1.3±0.3[+]
Metachromatic cells					
Adventitia	1.7±0.4	2.6±0.4	5.1±0.6[+]	4.6±0.6[+]	4.0±0.7[+]

Data are presented as mean±SEM of cell numbers per mm of basement membrane of airway. RSV6: respiratory syncytial virus 6 days prior to ovalbumin (OA). *: $p<0.05$, significant differences RSV/saline *versus* sham/saline; [+]: $p<0.05$; significant difference OA *versus* non-OA.

The interaction of allergen and specific IgE on the surface of sensitized mast cells and eosinophils can result in the activation of these cells and release of a variety of inflammatory cytokines and bronchoactive mediators [26]. In the present study, the virus-associated increases in titres of OA-specific IgG$_1$ antibody did not result in either enhanced AHR or increased airway inflammation by eosinophils or metachromatic cells in the RSV-infected, OA-exposed guinea-pigs. This finding is not surprising, since recent studies of knockout mice have provided evidence that allergen-induced eosinophilic inflammation and AHR can occur in the absence of interleukin (IL)-4 and OA-specific IgE [27]. Previous studies in mice which were genetically deficient in IL-5 or treated with an anti-IL5 antibody suggested that AHR may be directly associated with increased numbers of eosinophils and their products in the lungs [28, 29]. Accordingly, the lack of enhanced AHR in RSV-infected, OA-sensitized guinea-pigs could be related to the lack of virus-induced increases of eosinophilic airway inflammation in these animals.

In a recent report, RSV infection of mice, prior to OA sensitization, resulted in enhanced AHR, which was explained by a significant increase in lung eosinophilia, without an increase in specific IgE [13]. Although these animals were sensitized to OA, on day 21 postinfection when apparent resolution of changes induced by acute infection had occurred, no culture isolation of virus was performed at this time to determine whether RSV persisted long enough in the lungs to mediate the observed effects.

The present study also demonstrated that infectious RSV persisted in the lungs of guinea-pigs that were not exposed to OA, and this persistence was associated with increased AHR and increased airway eosinophilia. These findings extend previous observations of persistent RSV replication and histological evidence of increased granulocyte infiltrates in the guinea-pig lung [21]. They are consistent with other investigations that have documented long-term persistence of AHR and viral antigen in the lungs of RSV-infected guinea-pigs [30] and subacute increases in AHR after experimental rhinovirus type 16 infection of asthmatic patients [31]. The RSV-induced eosinophilia and AHR observed in the present study, however, did not potentiate the OA-induced increases in airway eosinophilia and AHR in RSV-infected, OA-sensitized animals. In this study, the total number of exposures of animals to OA and concentration of OA used in aerosols were intermediate between those used in previous studies of PIV-infected guinea-pigs and RSV-infected mice [12, 13]. The protocols used for exposure of

animals to OA could be important in explaining the results obtained by different groups of investigators.

The present results also highlight differences that are dependent upon the sequence of RSV infection and allergen sensitization. In a previous study, it was shown that when guinea-pigs were presensitized to OA by a protocol of repeated exposures to OA aerosols, a subsequent acute RSV infection potentiated AHR to a greater extent than was observed in uninfected, OA-sensitized animals or in nonsensitized, RSV-infected animals [18]. These results are consistent with the hypothesis that if a child is already allergically sensitized, the child might develop more severe symptoms of acute RSV bronchiolitis. In this situation, the RSV infection may represent a marker for existing allergy rather than acting as a trigger for the onset of allergic sensitization.

In summary, the results of this study show that increased ovalbumin-specific immunoglobulin G$_1$ antibody titres are indicative of allergic sensitization in guinea-pigs, but do not reflect either the extent of airway hyperresponsiveness or eosinophilic airway inflammation. In studying the role of the respiratory syncytial virus in the process of allergic sensitization, it is important to consider serological responses separately from the physiological or histological changes in the lungs of affected animals. This distinction may be relevant to the appropriate interpretation of intervention studies designed to prevent the sequelae of acute respiratory syncytial virus bronchiolitis in children.

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